PctA/ NarX protocol

1. **Extraction of Terminator part** from DNA 2016 extraction kit.
   Part B0015- 2016 kit, plate 3, 3F.
2. **Transformation to *E. coli* Top 10.**
   Glycerol stock + miniprep.
3. **Restriction of Terminator part with EcoRI & XbaI.**

Materials:

- Restriction Enzymes:___EcoRI & XbaI.___
- NEB buffer:___cutSmart____
- Molecular biology water

Procedure:

Set up the following reaction a described below:

Component	Volume
Buffer (X10)	4µl
DNA 2.5µg	33µl
Enzyme 1 -HF	1µl
enzyme 2 -HF	1µl
MB water	1 µl
Total volume	**40µl**

[B0015]= ng/µl
1µg (B0015)= 13.5 µl (~µl)
2.5µg (B0015)= 33 µl (~µl)
→ MB water=1 µl

1. Add appropriate amount of MB water to sterile 1.5ml tube.
2. Add restriction enzyme buffer to the tube. Vortex buffer before pipetting to ensure that it is well-mixed.
3. Add appropriate amount of DNA to be cut to the tube. Vortex DNA before pipetting to ensure that it is well-mixed.
4. Add 1µl of each enzyme
5. Place in a water bath at 37ºC for 1hr.
6. Purify digested inserts using gel extraction.

5. Run gel agarose (1%)- 5ul of the sample.

Goal- Purification inserts using gel before ligation.

Gel to 5ul of reaction →
Purification with Kit → Check Concentration of product (nano drop).
store at -20C or continue to next part.

6. Gibson assembly on 3 parts (Terminator, 2 parts of gBlock)

Gibson assembly of the products according to the lab manual

Calculation:

$$DNA\ Con'_{\left[\frac{Pmol}{\mu l}\right]} = \left(\frac{DNA\ Con'_{\left[\frac{ng}{\mu l}\right]} X1000}{Part\ size\ \ X650}\right)$$

Recommended Amount of Fragments Used for Assembly	
	2–3 Fragment Assembly
Total Amount of Fragments	0.02–0.2 pmols*X µl
Gibson Assembly Master Mix (2X)	10 µl
Deionized H_2O	10-X µl
Total Volume	20 µl***

control- vector only

Use 0.05pmol of plasmid and 0.15pmol of insert

Component	**Volume**
Plasmid (TT)	X
Insert (gBlock 1)	Y
Insert (gBlock 2)	Z

Gibson assembly mix	15µl
MBW	5-X-Y-Z
Total	**20µl**

Incubate samples in a thermocycler at 50°C for 60 minutes. Following incubation, store samples on ice or at –20°C for subsequent transformation.

7. Transformation

of the Gibson assembly products and control to TOP 10 (5ul)

8. plating on amp plates--> O/N

9. Verification

-PCR with primers to Mid of Tar only.

- Glycerol stock & miniprep

-Restriction of product with restriction enzymes

Materials:

- Restriction Enzymes SalI- HF, PstI- HF, ClaI)
- NEB buffer (Cutsmart Buffer)
- Molecular biology water

Procedure:
Set up the following reaction a described below:

For PctA-Tar

Component	**Volume**	**Volume**	**Volume**
Buffer (X10)	2µl	2µl	2µl
DNA up to 2µg	Xµl	Xµl	Xµl
PstI- HF	1µl	-	1µl
Sal I- HF	1µl	1µl	-
MB water	16-X µl	17-X µl	17-X µl
Total volume	**20µl**	**20µl**	**20µl**

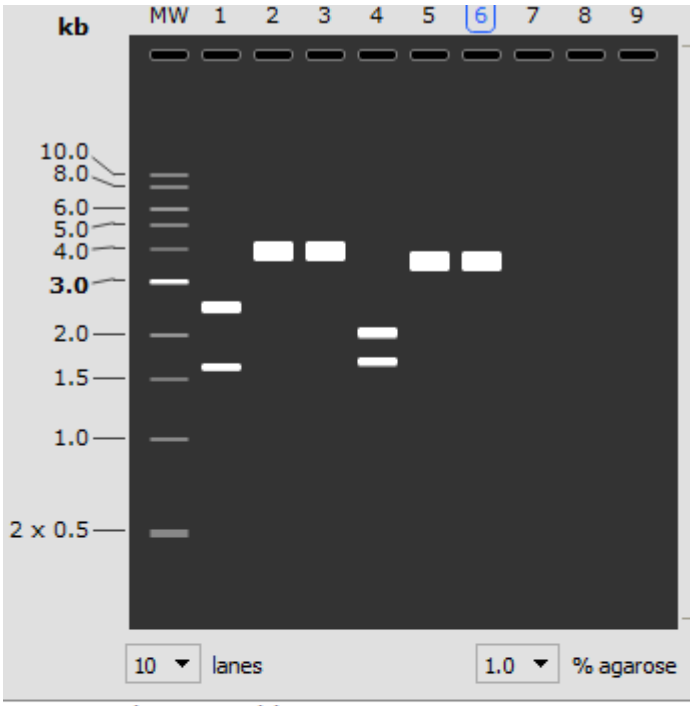
[cloned PctA-Tar]= Y ng/µl
1µg (cloned PctA-Tar)= µl (~µl)
2µg (cloned PctA-Tar)= µl (~µl)
→ MB water= µl

For NarX-Tar

Component	**Volume**	**Volume**	**Volume**
Buffer (X10)	2µl	2µl	2µl
DNA up to 2µg	Xµl	Xµl	Xµl
PstI-HF	1µl	-	1µl
ClaI	1µl	1µl	-
MB water	16-X µl	17-X µl	17-X µl
Total volume	**20µl**	**20µl**	**20µl**

[cloned NarX-Tar]= Y ng/µl
1µg (cloned NarX-Tar)= µl (~µl)
2µg (cloned NarX-Tar)= µl (~µl)
→ MB water= µl

- Gel
  Expected sizes:



MW: 1 Kb DNA Ladder

1: Cloned PctA+tar full plasmid
SalI + PstI
1. 2546 bp
2. 1644 bp

2: Cloned PctA+tar full plasmid
SalI
1. 4190 bp

3: Cloned PctA+tar full plasmid
PstI
1. 4190 bp

4: Cloned NarX+tar full plasmid
PstI + ClaI
1. 2108 bp
2. 1710 bp

5: Cloned NarX+tar full plasmid
ClaI
1. 3818 bp

6: Cloned NarX+tar full plasmid
PstI
1. 3818 bp

- Sending to sequence